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TITLE: Phase 0 Trial of Itraconazole for Early-Stage Non-Small Cell Lung Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of the proposed project is to determine the biologic effects of the drug itraconazole on lung cancer. Itraconazole is a well-tolerated and inexpensive (less than 10% the cost of recently developed molecularly targeted therapies) drug that has been in use for decades to treat fungal infections. Laboratory studies have shown that it also appears to block the growth of cancer through effects on tumor blood vessels (angiogenesis) and by targeting the Hedgehog developmental/survival pathway. In this clinical trial, 15 patients with early-stage lung cancer planned for surgical resection will receive approximately two weeks of itraconazole therapy. Before and during their treatment, they will undergo tissue and blood sampling in addition to magnetic resonance imaging (MRI) scans for biomarker analysis. At the time of surgery, resected tissue will be analyzed for similar biomarkers. These tissue, blood, and imaging biomarkers will be analyzed to determine itraconazole levels, effects on tumor blood vessels, effects on the Hedgehog pathway, and the correlation between each of these endpoints.

15. SUBJECT TERMS

Nothing listed

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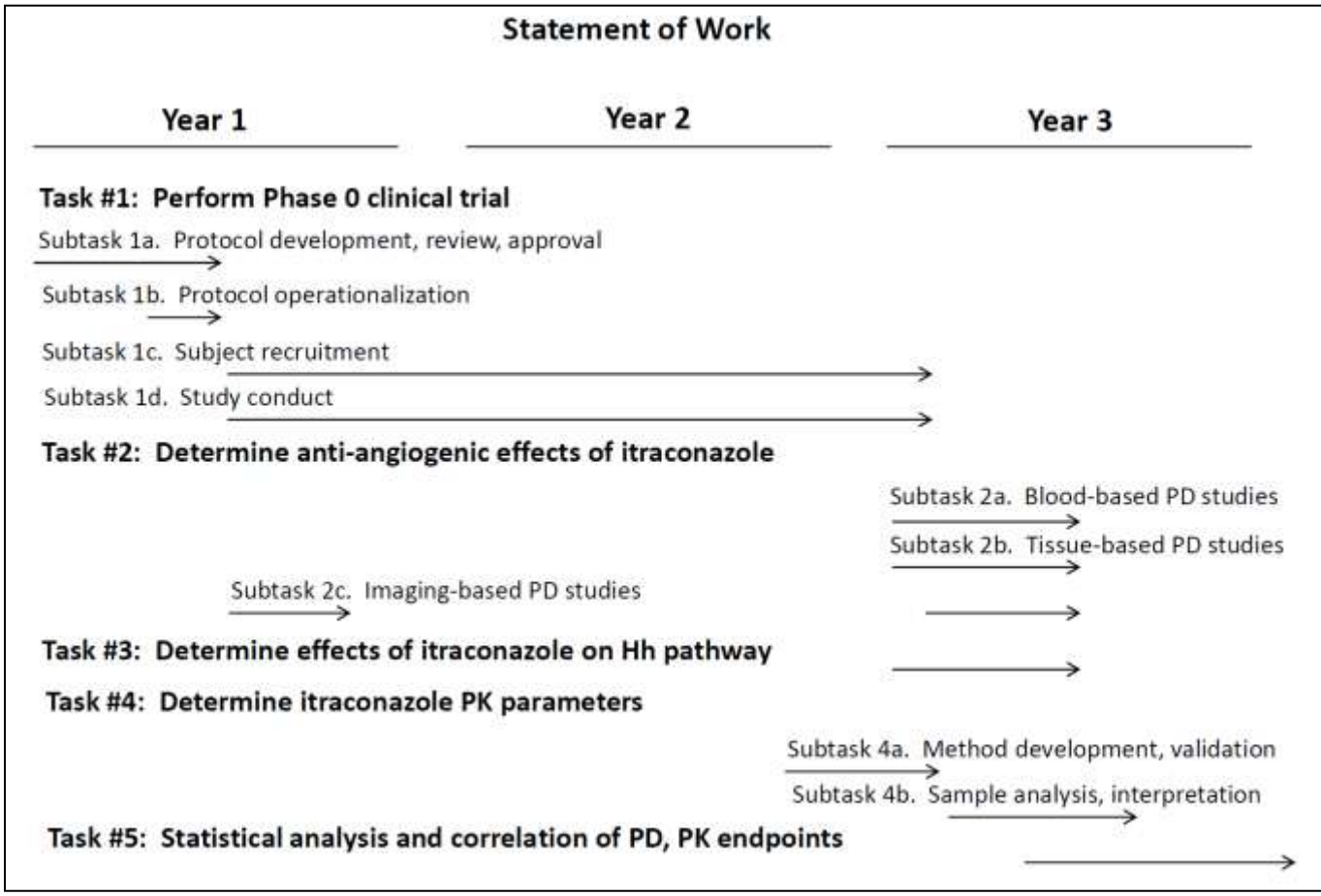
Background: Numerous drugs under development for lung cancer therapy are hindered by narrowly defined target populations, toxicity, and expense. Itraconazole—an antifungal agent in clinical use for decades—has shown promise as a lung cancer therapy with broad applicability, excellent safety, and a cost one-tenth that of many emerging lung cancer treatments. We and others have recently shown that itraconazole has anti-angiogenic properties, inhibits the Hedgehog (Hh) pathway (which plays a fundamental role in cancer stem cell biology), and results in tumor regression in preclinical models; inhibits the Hh pathway and tumor growth in human basal cell carcinoma; and possibly prolongs survival in advanced non-small cell lung cancer (NSCLC). Insight into itraconazole mechanism and biomarkers will provide essential guidance for further clinical development.
Objective/Hypothesis: **The overall objective of this phase 0 clinical trial is to determine the pharmacodynamic effects of itraconazole in early-stage NSCLC.** We hypothesize that itraconazole will inhibit tumor angiogenesis and will inhibit Hh pathway activation. Local itraconazole concentrations will be correlated with these effects. These hypotheses are supported by (1) studies demonstrating anti-tumor, anti-angiogenic, and Hh inhibitory effects in multiple preclinical cancer models; (2) a clinical trial developed by study team members in which itraconazole resulted in tumor regression and Hh pathway antagonism in basal cell carcinoma; and (3) a clinical trial in prostate cancer in which itraconazole-medicated Hh pathway inhibition was dose-dependent.
Specific Aims: *(Aim 1)* **Determine effects of itraconazole on tumor angiogenesis.** Pre- and post-treatment tissue samples, peripheral blood samples, and imaging studies will be analyzed for multiple angiogenic biomarkers and compared. *(Aim 2)* **Determine effects of itraconazole on the Hh pathway.** Pre- and post-treatment tissue samples and skin biopsies will be analyzed for GLI1, SHH, and PTCH1 levels and compared. Proliferation (Ki67), apoptosis (cleaved caspace 3), and resistance (SMO gene mutations, GLI2 and CCND1 copy number, PI3K-mTOR pathway activation) markers will also be quantitated and compared to effects on Hh pathway components. *(Aim 3)* **Determine the effect of itraconazole pharmacokinetics (PK) on the pharmacodynamic profile of itraconazole.** On-treatment serum, tumor tissue, and skin biopsies will be analyzed for itraconazole concentrations. Plasma and tissue analyses of itraconazole levels will be determined using a validated ultraperformance liquid chromatography (UPLC)-mass spectroscopy (MS)/MS method.
Study Design: This is a 15-patient, single-arm phase 0 clinical trial. Patients with early-stage NSCLC (any histologic subtype) will undergo baseline tumor biopsy, skin biopsy, blood sampling, and MRI studies (dynamic contrast enhanced [DCE], diffusion weighted imaging [DWI], and arterial spin labeling [ASL]. Participants will then be treated with itraconazole 600 mg orally daily for 7-10 days followed by repeat skin biopsy, blood sampling, and MRI studies, followed by surgical resection. Baseline and post-treatment biomarkers will be compared using t-tests, Wilcoxon signed rank tests, and nonlinear mixed effect models.
Clinical Impact: Results of this study will inform patient selection, treatment regimens, and biomarkers in future studies of itraconazole. The low cost, existing FDA approval, and longstanding safety record of itraconazole will permit subsequent clinical trials examining the repurposing of this drug as a lung cancer therapy to proceed rapidly.
Project Status: In the first year of the award period, the following has been accomplished: (1) development of a full clinical trial protocol, (2) approval by local Institutional Review Board (IRB) and Department of Defense (DOD) review committee, (3) assembly of local Data Safety and Monitoring Committee (DSMC) for trial, (4) coordination of clinical, radiology, laboratory, and pathology resources to implement correlative study procedures, (5) activation of study protocol at UT Southwestern Medical Center and Parkland Health and Hospital Systems, (6) enrollment of two patients on protocol with no apparent adverse events related to study therapy or procedures, (7) performance of certain imaging and laboratory correlative studies for the enrolled patients.

1. **INTRODUCTION:** The objective of the proposed project is to determine the biologic effects of the drug itraconazole on lung cancer. Itraconazole is a well-tolerated and inexpensive (less than 10% the cost of recently developed molecularly targeted therapies) drug that has been in use for decades to treat fungal infections. Laboratory studies have shown that it also appears to block the growth of cancer through effects on tumor blood vessels (angiogenesis) and by targeting the Hedgehog developmental/survival pathway. In this clinical trial, 15 patients with early-stage lung cancer planned for surgical resection will receive approximately two weeks of itraconazole therapy. Before and during their treatment, they will undergo tissue and blood sampling in addition to magnetic resonance imaging (MRI) scans for biomarker analysis. At the time of surgery, resected tissue will be analyzed for similar biomarkers. These tissue, blood, and imaging biomarkers will be analyzed to determine itraconazole levels, effects on tumor blood vessels, effects on the Hedgehog pathway, and the correlation between each of these endpoints.

2. **KEYWORDS:** lung cancer; itraconazole; repurposing; Hedgehog pathway; stem cell; angiogenesis; neoadjuvant; pharmacodynamic; pharmacokinetic.

3. **OVERALL PROJECT SUMMARY:** Year 1 Progress Report. Original Statement of Work printed below.



Task 1: Perform Phase 0 Clinical Trial

Subtask 1a: Protocol Development, Review, Approval

A full protocol was developed by Study Chair and PI Dr. David Gerber, in conjunction with UT Southwestern faculty member Dr. Lorraine Pelosof. The full protocol and informed consent form were approved by the UT Southwestern Harold C. Simmons Comprehensive Cancer Center Protocol Review and Monitoring Committee (PRMC) on January 22, 2015. The full protocol and informed consent form were approved by the UT Southwestern IRB on March 17, 2015 (STU 122014-038). Provisional approval was received by the Office of Research Protections (ORP) U.S. Army Medical Research and Materiel Command (USAMRMC) (Proposal Log Number LC130650, Award Number W81XWH-14-1-0540, HRPO Log Number A-18295) on May 19, 2015. The protocol was activated at UT Southwestern Medical Center and Parkland Health and Hospital System in June 2015, approximately two months after our initial goal of April 2015.

Subtask 1b: Protocol Operationalization

During the first 6 months of the award period (October 2014-April 2015), I convened regular meetings with all involved parties. This included representation from the Clinical Research Office; Departments of Radiology, Thoracic Surgery, Pulmonary Medicine, Hematology-Oncology; Texas Tech School of Pharmacy; Hamon Center for Therapeutic Oncology Research; and the Biomarker Research Core.

We established Standard Operating Procedures for all study procedures. We ordered all required supplies for study procedures.

Subtask 1c: Subject Recruitment

Recruitment began in earnest in July 2015. As this is the first window-of-opportunity trial in lung cancer to be performed at UT Southwestern Medical Center, we were required to educate and encourage our colleagues in the Thoracic Surgery Program. Patients with early-stage lung cancer generally are not evaluated by medical oncology prior to surgical resection. We met with these physicians, mid-level providers, and clinic nurses. We prepared "cheat sheets" for these individuals that contained contact information and a basic description of study eligibility. We pre-screened patients referred to the thoracic surgery faculty. We reminded clinicians of the trial at weekly thoracic oncology tumor boards.

After an initial period of inactivity, we witnessed study accrual increase substantially this fall. During the 6 weeks leading up to this progress report, a total of 4 patients were referred to the trial. One was found to be ineligible. One is currently in screening. Two have completed the trial.

Based on widespread awareness of the trial, growing enthusiasm for the project, feasibility of study procedures, and lack of apparent adverse events from study therapy or procedures, we anticipate that accrual will continue at this rate. We therefore project that we will meet our initial accrual completion goal of early during Year 3 of this award.

Subtask 1d: Study Conduct

Two patients have been enrolled as of this annual report. Their demographic and tumor information are listed in the table below. All patient identifiers have been removed.

Age	Sex	Race	Tumor Stage	Histology
63	Male	Caucasian	T1bN0M0 Stage IA	Undifferentiated carcinoma, favor Large cell
63	Female	Caucasian	T1aN0N0 Stage IA	squamous cell carcinoma

As described in the original proposal, these subjects underwent study-related MRI scans, skin biopsies, blood tests, treatment with itraconazole, and surgical resection. To date, there have been no technical difficulties encountered with any clinical procedures.

Task 2: Determine anti-angiogenic effects of itraconazole

Subtask 2a: Blood-based PD studies

As described in the original Statement of Work, this subtask is planned to be performed during Year 3 of the project. Nevertheless, once we have enrolled approximately 5 patients, we will batch their samples and perform an initial run of the multiplex cytokine assay to confirm that sample collection and processing techniques are adequate.

Subtask 2b: Tissue-based PD studies

As described in the original Statement of Work, this subtask is planned to be performed during Year 3 of the project. Nevertheless, we are currently in the process of performing the proposed immunohistochemical tests on clinical biospecimens from the first two patients to confirm that sample collection and processing techniques are adequate.

Subtask 2c: Imaging-based PD studies

We have performed research MRI scans on enrolled patients. Sample images (**Figure 1**) demonstrate feasibility of our techniques. We do not anticipate any modification to our approach.

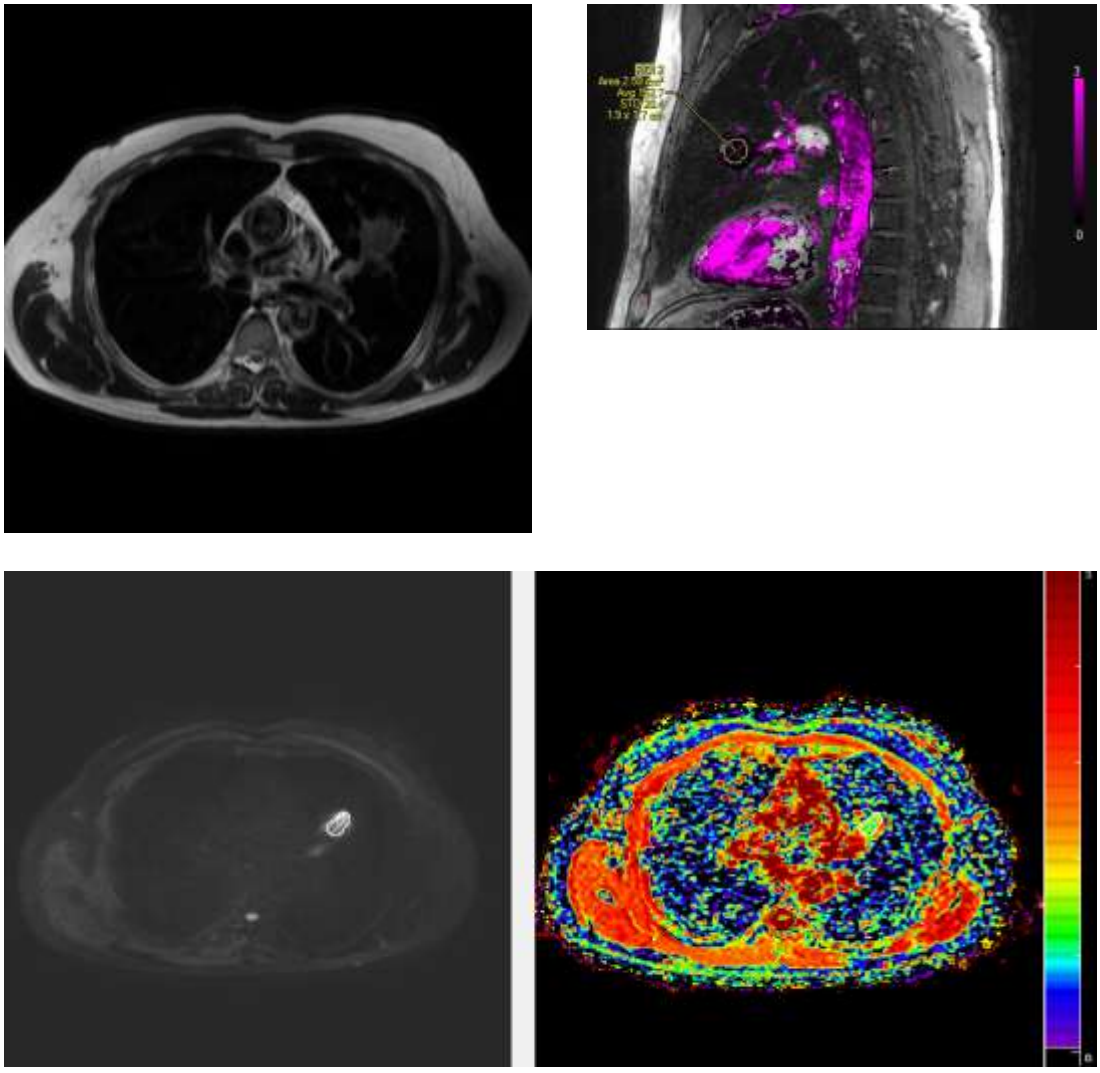


Figure 1. Sample baseline MRI images from enrolled patient. *(Top left)* T2 MRI; *(Top right)* DCE MRI; *(Bottom)* DWI MRI

Task 3: Determine effects of itraconazole on Hh pathway

Although not planned until Year 3 of the project, we are initiating these studies on tumor tissue and skin biopsy specimens from the initially enrolled patients to confirm adequacy of collection and processing techniques. As of this report, we have performed mRNA analysis of target biomarkers in serial skin biopsies from a single patient (**Figure 2**). Tumor tissue analysis is forthcoming.

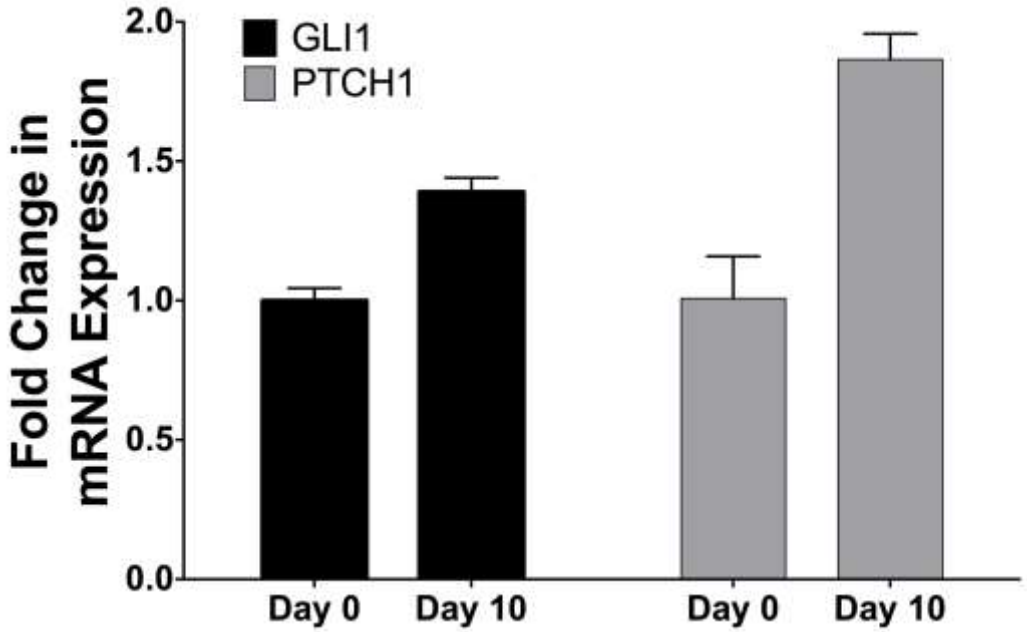


Figure 2. mRNA expression of GLI1 and PTCH1 from serial skin biopsies from enrolled patient.

Task 4: Determine itraconazole PK parameters

Subtask 4a: Method development, validation

Planned for Year 3 of project.

Subtask 4b: Sample analysis, interpretation

Planned for Year 3 of project.

Task 5: Statistical analysis and correlation of PD, PK endpoints

Planned for Year 3 of project.

4. KEY RESEARCH ACCOMPLISHMENTS:

Nothing to report.

5. CONCLUSION:

Nothing to report.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Nothing to report.

7. INVENTIONS, PATENTS AND LICENSES:

Nothing to report.

8. REPORTABLE OUTCOMES:

Nothing to report.

9. OTHER ACHIEVEMENTS:

Nothing to report.

10. REFERENCES:

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Teglund, S. and R. Toftgard, *Hedgehog beyond medulloblastoma and basal cell carcinoma.* Biochim Biophys Acta, 2010. **1805**(2): p. 181-208.

11. APPENDICES: None

NOTE:

TRAINING OR FELLOWSHIP AWARDS: For training or fellowship awards, in addition to the elements outlined above, include a brief description of opportunities for training and professional development. Training activities may include, for example, courses or one-on-one